REMARKS

The claims have been amended to clarify the claimed subject matter. More particularly, the claims have been amended to reflect that the present claimed subject matter is directed to an improved method of quantifying proteins by directly detecting the signal produced by a labeled ligand that binds directly to the target protein. Accordingly, the method does not utilize a secondary binding entity that binds to the ligand to amplify the signal, nor does it require an enzymatic or chemical reaction to detect the labeled ligand.

Claims 1 and 13 have been amended to specify that the claimed method is used to determine the concentration of low concentration proteins through the use of a labeled ligand that binds directly to the target protein, wherein the binding is directly measured for determining the concentration of the protein in the sample. Support for the claim amendments is found on page 10, lines 16-21 and on page 14, lines 25-30. Claims 2, 17 and 18 have been amended to specify that the labeled ligand is fluorophor-conjugated antibody. Support for this amendment is found on page 3, lines5-7, page 10, line 19 and original claim 19. Claim 3 is amended to specify that the immunological detection of the protein is conducted base solely on fluorescence detection. Support for the amendment is found on page 9, lines 5-17. Support for the amendment to claim 8 is found on page 12, lines 17-22. Support for new claims 30 and 31 is found on page 12, lines 8-9.

Elections/Restrictions

The Examiner notes applicant's election, with traverse of the Group II invention (claims 13-21). The Examiner states that since the supposed error in the restriction requirement was not detailed, the election has been treated as an election without traverse.

As detailed below, the present invention is directed to an improved general procedure for quantitating proteins. As such, the claimed method works independent of the targeted protein to be quantitated. As noted repeatedly throughout the specification (see for example page17, lines 8-11) the quantification of the P450 protein was used as a simple example of operability of the claimed methods. Accordingly, applicants respectfully request the rejoinder of the original Group I claims with the Group II claims, and that claims 1-21 be examined in the present application. The examination of the generic claims with the present P450 species claims is believed not to constitute an additional burden to the Examiner since the patentability of claims 1-12 and 13-21 rest on the same principle.

Claim Rejections 35 USC § 112

Claim 16-17 stand rejected for the use of a trademark to identify an element of the claim. Claim 16 has been amended to specify the amino acid sequence of the FLAG peptide. The FLAG peptide (and its corresponding antibodies) is well known to those skilled in the art and is commercially available from Sigma-Aldrich Sigma-Aldrich Corp. (St. Louis, MO USA). Therefore, applicants respectfully submit the addition of the amino acid sequence does not constitute new subject matter. The amendment of claims 16 and 17 are believed to fully addresses the Examiner's concerns and applicants request the withdrawal of the rejection based on 35 USC § 112.

Claim Rejections 35 USC § 103

Claims 13-14, 16, 18, 19 and 21 stand rejected as being unpatentable over Ro et al., in view of Kay. Applicants respectfully traverse.

The primary Ro reference is directed to investigating the subcellular localization of C4H in transgenic Arabidopsis plants. To this end, C4H was modified to contain a FLAG epitope and an anti-FLAG antibody was used as part of a traditional multi-component, multiantibody signal amplification system to identify the P450 in a western blot, as shown in Figure 5 of their paper. Thus Ro fails to teach or suggest a system that directly detects a labeled ligand/antibody, wherein the labeled antibody directly binds to the target protein (i.e. C4H). Although Ro discloses the use of an anti-FLAG antibody that directly binds to the C4H protein, that antibody is not labeled. Ro discloses the use of a secondary "mouse horseradish peroxidase-conjugated anti-mouse antibody that binds to the first antibody. However this secondary antibody does not constitute a "labeled ligand that directly binds to the epitope" as required by the claims.

This difference between the prior art methods and the present claimed method is significant because, as stated at page 1, line 27 through page 2, line 3 of the specification, the amplification of the signal obtained through the use of a secondary antibody also amplifies errors present in the system. Accordingly, the prior art methods, while useful for detecting proteins, are not reliable for protein quantification.

Furthermore, applicants note that the system disclosed by Ro uses chemiluminesence as the detectable signal, not fluorescence. The primary antibody disclosed by Ro carries no fluorescent tag and they do not teach or suggest the use of a phosphorimager to detect a signal from their protein. Again, the significance of this difference is that Ro teaches

amplification of the signal twice, once by the anti-mouse secondary antibody, and again by a chemiluminesent reaction in which light is emitted from a chemical reaction, not from fluorescent excitation by an external energy source. Accordingly, because of the above differences, the western blot methodology disclosed by Ro is only qualitative in nature, while the technique described in the present invention is quantitative.

The secondary Kay reference fails to supplement the inadequacies of the primary Ro reference. While Kay does teach that antibodies can be labeled with a fluorescent chromophore, there is no teaching or suggestion that such techniques could be used in western blot analysis to quantitate low levels of proteins. The technology described in the present application illustrates a new and novel use for chromophore-labeled antibodies that is far beyond the scope of Kay's patent, and was never envisioned by Kay. The examples provided in Kay demonstrate his vision that this technology was a qualitative (not quantitative) approach for the identification of disease-related micro-organisms and macromolecules including DNA, RNA, bacteria, fungi, protozoans, etc. (Column 1, lines 3-10, and Column 1, lines 49-63). In Column 4, lines 28-29 Kay states that "The operative solution is used in the same manner as are presently known labeled antibody solutions." As a person skilled in the art of the protein sciences in 1974, Kay surely understood that the "presently known labeled antibodies" of the time only allowed for qualitative testing. In contrast, one of the primary strengths of the presently claimed technology is that it allows quantification of the proteins.

Furthermore, the limits of detection described by Kay are "500 organisms per milliliter" (Column 1, lines 64-72). In contrast, the technology described in the present application has a demonstrated limit of quantization with a minimum of a 10:1 signal to noise ratio of 0.8 picomoles (10^{-12} moles) of protein per assay. The limit of detection for the technology described in applicant's application is in the femtomolar range (10^{-15} moles) of protein per assay. Anyone possessing ordinary skill in the art of protein science would recognize that the limits of detection possible using the technology described in the present application are many orders of magnitude lower (better) then described by Kay. Additionally, anyone possessing ordinary skill in the art of protein science would recognize the value of protein quantization of fractions of picomoles of protein per assay with a demonstrated high signal to noise ratio as desirable over the technology described by Kay.

The cited references for all their combined teachings fail to teach or suggest the use of a chromophore labeled ligand that directly binds to a target protein to serve as the sole detectable signal for quantitating the amount of protein present in a sample. Accordingly, applicants respectfully request the withdrawal of the rejection of claims 13-14, 16, 18, 19 and 21 as being unpatentable over Ro et al., in view of Kay.

Claims 15 and 17 stand rejected as being unpatentable over Ro et al., in view of Kay, further in view of Einhauer & Jungbauer. Applicants respectfully traverse.

Applicants note that claims 15 and 17 ultimately depend from independent claim 13. The Einhauer & Jungbauer reference is devoid of any suggestion regarding the use of chromophore labeled antibodies to quantify proteins. Accordingly, the Einhauer & Jungbauer reference fails to supplement the deficiencies of the Ro and Kay references with regards to claim 13, and therefore claims 15 and 17 are patentable as well. Accordingly applicants request the withdrawal of the rejection of claim 15 and 17 as being obvious over those references.

Claim 20 stands rejected as being unpatentable over Ro et al., in view of Kay, further in view of Amersham Biosciences. Applicants respectfully traverse.

In 1999 Amersham Biosciences was marketing kits for western blot protein detection called the ECL Western and ECL Plus Western. The kit includes a secondary antibody that amplifies the detection signal, then a tertiary chemical reaction involving chemiluminescense to further amplify the signal and make the signal detectable by film or by other photographic techniques. Accordingly, similar to the Ro reference, Amersham teaches the detection of proteins using indirect means. The ECL technology uses a secondary antibody to amplify signal (and noise/error) while the technology described in the present application does not. Furthermore, the ECL technology uses a tertiary chemical reaction to amplify the signal by chemiluminesence while the technology described in the present application uses no chemical reactions to produce products. In the present claimed method, fluorescence, not chemiluminesence is used for signal detection.

The Amersham reference cited by the Examiner observes that the biproduct(s) of the ECL chemiluminescent reaction are fluorescent, and can be detected by a Molecular Dynamics (now GE) Storm phosphorimager. Application note the cited Amersham reference (80-6443-83) goes on to describe how the Storm phosphorimager, which is intended to detect radioactively-labeled compounds, can function as a dual-use instrument to also detect

fluorescent compounds on a western blot. However, there is simply no suggestion within the Amersham reference that the techniques described therein could be used to detect a non-amplified fluorescent signal. Furthermore, there is no teaching or suggestion in that reference that a fluorescent chromophore-labeled antibody that binds directly to the target protein could be used to quantitate proteins present on a western blot.

The ECL fluorescence detection disclosed by Amersham relates to the detection of a fluorescent molecule generated by a chemical reaction that is not covalently bound to any other component of the detection reagents. The many variables associated with this chemical reaction (reagent age, dilution, temperature, human error, etc.) make this approach far less suitable for reproducible protein quantization in comparison to the technology described in the present application. Accordingly, the methods described in the Amersham reference are subject to the same disadvantages as the methods described in Ro.

Although the Amersham reference may suggest that a phosphor autoradiography imager could be used to detect an amplified fluorescent signal being produced by a secondary antibody that binds to a primary antibody, there is no suggestion of applicants direct binding and detection of a labeled ligand to a target protein. Accordingly, applicants respectfully submit the Amersham reference fails to teach or suggest the claimed invention, and therefore request the withdrawal of the rejection based on this reference.

The cited references for all their combined teachings fail to teach a method of quantating a protein wherein a chromophore labeled antibody directly binds to a target protein and provides the sole signal for detection and quantification of the protein. The foregoing claim amendments and remarks are believed to fully respond to the Examiner's rejections. The claims are believed to be in condition for allowance. Applicants respectfully request allowance of the claims, and passage of the application to issuance.

Respectfully submitted,

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